REMARKS

Amendments to the specification

The description of the deposit corresponding to the rituximab antibody is revised at paragraph 0220 to correctly characterize the deposited material. The amendments reflect the disclosure in U.S. Patent No. 5,736,137, which is incorporated by reference into the disclosure of this application and specifically cited at paragraph 0220. In particular, the deposit is not an antibody-producing transfectoma, but an *E. coli* cell line carrying a vector, TCAE-8, that includes the complete coding sequences for the C2B8 (rituximab) antibody. The '137 patent sets forth the complete DNA sequence of this vector, including the heavy chain and light chain coding sequences at Figure 3, and the corresponding amino acid sequences of the variable domains of the heavy chain and light chain, respectively, at Figures 4 and 5.

The second sentence of paragraph 0270 is corrected to refer to a dosage of 375 mg/m^2 instead of 375 mg/kg. The correction is supported by express disclosure in the working examples, *e.g.*, at paragraph 0360.

At paragraph 0280, the identifying information for the deposited hybridoma that produces the murine monoclonal antibody, 2B8, is added. The term "cell line" is also replaced by "hybridoma," to more completely characterize the deposited material. The added information is set forth at col. 32 of U.S. Patent No. 5,736,137, which is incorporated by reference in the disclosure of this application as filed.

The disclosure as filed incorrectly identified the CHOP regimen at paragraph 0290 as "immunotherapy." As would be immediately understood by one skilled in the art, CHOP (a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone, as described in the specification) is a chemotherapeutic regimen. The specification is revised to correct this obvious error.

At paragraph 0310, clinical data in Example 1 that were amended in the response filed on 29 August 2000 are replaced by the data presented in the application as filed. The data serve to quantitate the depletion of B cells in patients in response to rituximab therapy. For the reasons stated in the earlier amendment, it would have been obvious to one of skill that the some of the

numerical values at page 9 of the original specification could not be correct. However, the original data are restored in the present amendment to moot any question regarding proper basis for the earlier correction. The text of this paragraph, which also appears in priority application 60/107,658, corresponds closely to a meeting abstract published shortly after the filing of the priority application as J.C. Byrd *et al.*, *Blood* 92(10, suppl. 1): 106a, abst. no. 432 (1998) (copy attached), clearly describing the same clinical trial. The previous amendments appear to correspond to reported data, but in any event, the numerical data are not necessary to support the claimed invention.

At paragraph 0320 (which is split in this amendment from paragraph 0310 solely for readability), the original text from page 10, line 2, concerning a stepped-up dosing regimen is corrected. This text was amended in the response filed on 29 August 2000 to correct typographical errors. Two changes were made in the previous amendment: first, the initial dose of 100 mg was changed to 100 mg/m^2, and second, the reported timing of the second administration was changed from day 1 to day 2. The corresponding sentence from the Byrd abstract, discussed in the paragraph above, is reproduced here:

> Two subsequent patients with CLL have been treated at our institution with high blood tumor counts utilizing stepped up dosing (100 mg on day 1 followed by completion of the remaining therapy on day 2) with demonstrated efficacy, thombocytopenia but minimal infusion-related toxicity.

Thus, the first change appears to be an erroneous revision, whereas the second faithfully reflects the text of the meeting abstract.

The original text at paragraph 0320 that reports the initial dose as 100 mg, rather than mg/m^2, is restored by this amendment. There is nothing in the application as filed that would have suggested to one of skill that "100 mg" was an error. Taking the meeting abstract as accurate, the application as executed by the inventors in fact describes the clinical trial that was actually performed.

The phrase at paragraph 0320 that describes the timing of the administration on day 2 is replaced by the original reference to day 1. Applicant notes that in the referenced trial, the second administration occurred on day 2, rather than day 1. The information regarding the

timing of the exemplified stepped-up dosing schedule is not necessary to support the claimed invention.

A reference to a publication by McLaughlin *et al.* is revised at paragraph 0360 to reflect the original text at page 12, line 11, citing "McClaughlin et al, KOO, Vol. 14, 1998." The misspelling of the lead author's name is an obvious error, and one of skill would have understood that "KOO" is not a common abbreviation for any journal. The replacement citation, a paper published at *J. Clin. Oncol.* 16(8): 2825-33 (1998), in fact discloses the treatments described in the specification. However, to moot any question about the correctness of the replacement citation, the original text is restored. In any case, the McLaughlin reference is not incorporated as part of the disclosure; the disclosure of the *JCO* paper was known to those skilled in the art when the application was filed; and the citation is not necessary to support the claims.

The reference to "β_2 immunoglobulin" at paragraph 0370 was present in the specification as filed. As one of skill would have recognized that this clinical parameter could only be meant to refer to β_2 <u>micro</u>globulin levels, the obvious error is corrected by this amendment.

One of skill would have immediately recognized that the reference in paragraph 0400 to "Ficon Hypaque" separations is an obvious error, and that the correct reference is "Ficoll Hypaque." This error is corrected, and the trademark, FICOLL®, is properly noted together with the generic terminology.

In the Office action mailed on 29 February 2000, the examiner objected to the presence in the specification of the URL, "http://www.cancernetwork.com," because it was presented in the form of a hyperlink that would be executable if the text were rendered on a web page. In the amendment filed on 29 August of that year, the reference to the website was replaced with citations to three references containing equivalent information. The original reference to the website (which is not incorporated by reference) is restored by the present amendment at paragraph 0420 to moot any question concerning basis in the original disclosure for the cited information. The address of the website is printed in a form that will not be rendered as an executable hyperlink, in conformance with M.P.E.P. § 608.01.

The amendments also address various informalities throughout the specification. The priority claim (paragraph 0010) is amended to include the filing date of the priority application. The international nonproprietary name (INN) of the chimeric antibody in the RITUXAN® drug product, rituximab, is included at every reference to the product. Other changes provide spelled-out words at the first occurrences of abbreviations and, in some cases, replace abbreviations that are well known in the art of clinical oncology. Grammatical and typographical informalities are also rectified.

The title and abstract are revised to reflect the amended claim set.

None of the amendments to the specification introduces new matter into the disclosure.

Amendments to the claims

The new claims are supported in the disclosure as filed as follows, with reference to the paragraph numbers in the substitute specification. The claims add no new matter to the disclosure.

Treatment of chronic leukocytic leukemia (CLL) is supported throughout the disclosure. The range of 0.001 to 30 mg/kg and narrower included ranges (claims 30-32, 56-58) are supported, *e.g.*, at paragraph 0250. A dosage of 375 mg/m^2 (claims 34, 59) is described, *e.g.*, at paragraphs 0270 and 0360. The range of 500 to 1500 mg/m^2 and specific intermediate doses (claims 34-38, 60-64) are supported, *e.g.*, at paragraph 0370. Applicant notes that this dosage range would cover at least the administration of a fixed dose comprising, *e.g.*, about 900 to about 2700 mg of antibody.[1]

Treatment of relapsed (claims 39, 65) and refractory (claims 40, 66) CLL is described, *e.g.*, at paragraph 0430. Fludaribine-refractory patients (claims 41, 67, 94) are described, *e.g.*, at paragraph 0420. The use of chimeric, humanized, and human antibodies and antibody fragments (claims 42-46, 68-72) are described, *e.g.*, at paragraph 0150. The use of rituximab (claims 43, 69) is described throughout the disclosure.

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[1] Based on conversions from mg/m^2 to mg dosages for an "average" 70 kg, 67 inch human.

The recitation of repeated administration (claims 47-52, 73-78) is supported, *e.g.*, by the description of weekly, biweekly, and monthly administration (claims 49-52, 75-78) at paragraph 0260. The specific dosing schedule of claims 50 and 76 is described, *e.g.*, at paragraph 0270. The recitation of a stepped-up dosing schedule, (claims 48, 74) is described, *e.g.*, at paragraphs 0270 and 0320. The recitation of parenteral and intravenous administration (claims 53-54, 79-80) are supported, *e.g.*, at paragraph 0230.

Combination treatments with chemotherapy (claim 55) are described, *e.g.*, at paragraphs 0140 and 0410-0440. Concurrent therapies (claim 81) are described, *e.g.*, at paragraph 0440. Specific chemotherapeutic agents recited in claims 82-93 are identified, *e.g.*, at paragraphs 0290 and 0440.

The claims are now directed specifically to the treatment of CLL using any unlabeled anti-CD20 antibody that is administered in an amount that is effective to treat the CLL. The new claims also differ from the claims they replace in that the amount of anti-CD20 antibody administered to the patient is required to be "effective to treat the chronic lymphocytic leukemia," instead of "effective to achieve a reduction in circulating tumor cells." One of skill in the art of clinical oncology would understand that effective treatments of CLL include, but are not necessarily limited to, those assessed with respect to a reduction in circulating tumor cells.

The limitation requiring a reduction in circulating tumor cells was added to the claims in the amendment filed 29 August 2000 in response to a rejection under 35 U.S.C. § 112, second paragraph, set forth in the first Office action mailed on 29 February 2000. Original claim 1 read:

> A method of treating a hematologic malignancy associated with high numbers of circulating tumor cells by administering a therapeutically effective amount of an anti-CD20 antibody or fragment thereof.

Applicant understands that the basis for the rejection under § 112, second paragraph, was that the claims then pending failed to specify what the "effective amount" should be effective to do, and thus that one of skill would not understand what method steps would be required to effect the recited "method of treating." The new claims are free of the ground of rejection previously stated.

Rejection under 35 U.S.C. § 102(e)

Claims 4, 5, 8, 11, 13, 14, 16, and 28 were rejected under § 102(e) as anticipated by Kaminski, U.S. Patent No. 6,090,365. For the following reasons, new claims 29-94 are patentable over the reference.

The discussion of CLL in the Kaminski patent is insufficient to anticipate the present claims. Although the patent arguably suggests the applicants' desire to include treatments of CLL within the scope of the purported invention, there is in fact no description and no exemplification of a specific treatment of any CLL patient using any anti-CD20 antibody. The mere mention of CLL among several B cell tumors in the Kaminski patent is not tantamount to an enabling disclosure of any embodiment that meets all the limitations of the claims.

Applicant incorrectly stated in the response filed on 2 May 2005 that CLL was noted only once in the Kaminski patent. In fact, it is mentioned in four passages. None of these passages, however, describes a therapeutic treatment for CLL using an anti-CD20 antibody.

- The passage at col. 2, lines 5-11 and 29-34, describes prior art treatments of CLL patients using an antibody, T101, directed against an uncharacterized T cell antigen. Plainly, such disclosure does not describe or suggest the treatment of CLL patients using a B cell-directed therapy such as administration of an anti-CD20 antibody.

- At col. 4, lines 41-63, CLL is mentioned in the middle of a discussion of antibodies that recognize tumor cell antigens *other than* CD20. In particular, the passage contemplates the use of the antibodies B4, which recognizes the CD19 antigen, and B2, which recognizes the CD22 antigen, and it teaches that both of those antigens have been observed on CLL cells. This passage might suggest that therapies directed at CD19 or CD22 could be explored for treating CLL, but it says nothing about whether a treatment directed at CD20 could be used to treat that disease.

– The paragraph at col. 6, lines 10-18, summarizes all of the antibodies and all of the tumors discussed in the Kaminski patent application, and it expresses the applicants' desire to include all of those within "the invention." The entire paragraph is reproduced here:

> Also, the invention is not limited to the CD19 and CD20 antibodies. Rather, the invention encompasses the use of antibodies which are identify antigens associated with cells of the B cell lineage to treat cancers which are clonal from such cells. Examples of such antibodies are B2, B3, B4 (HD-237), and J5, in addition to B1. Examples of such cancers are ALL, CLL, Hairy Cell leukemia, and chronic myeloblastic leukemias in a blast crisis stage, in addition to lymphomas.

This paragraph cannot be fairly read to teach that *all* of the enumerated antibodies can be used to treat *all* of the recited diseases. Indeed, the passage that includes col. 4, lines 41-63, discussed above, would be inconsistent with such a reading. That passage teaches that different tumors express different antigens, and antibodies directed against different antigens exert therapeutic effect by divergent mechanisms. The passage reproduced above does not describe, nor does it suggest, using antibodies directed against any specific B cell antigen to treat any specific B cell tumor.

– Finally, CLL is mentioned in the paragraph at col. 8, lines 12-47. This paragraph describes the clinical reactivity profile of the B1 antibody. Specifically, it teaches that the B1 antigen (*i.e.*, the antigen recognized by the B1 antibody, which is CD20) is present on a variety of normal B cells. It further states that the B1 antigen "is also expressed on tumor cells isolated from … greater than 95% of patients with B cell chronic lymphocytic leukemias (CLL) … ." Thus, this passage teaches that CD20-positive cells are present in most CLL patients. However, it does not indicate whether CD20 is present on a high percentage of tumor cells in CLL patients, or at what levels. The passage does not purport to suggest that CD20 is an appropriate target for CLL therapy.

In sum, the Kaminski patent teaches – at most – that the CD20 antigen is found on at least some tumor cells in most CLL patients, and that, as a general concept, B cell tumors could conceivably be treated using B cell-directed antibodies. These generic disclosures, even if supported by speculation about what the applicants in the Kaminski patent might have contemplated, do not provide the sufficient or enabling teachings required to anticipate under § 102.

New claims 29-94 further differ from the disclosure of the Kaminski patent as they require the use of an unlabeled anti-CD20 antibody in an amount effective to treat CLL. Kaminski expressly teaches that the therapeutic benefit in the procedures it describes are due to the administration of radiolabeled B1 antibody. For example, the Brief Description of the Invention at cols. 3-4 sets forth four therapeutic approaches of the purported invention, all providing for therapeutic doses of radiolabeled antibody. With respect to one of these approaches, the patent teaches at col. 3, lines 54-57:

> A third method using B1 antibody comprises administering to a patient a large amount of an unlabelled antibody, which can be B1 but can also be other antibodies, prior to administration of a therapeutic dose of labeled B1 antibody.

As the patent explains more fully at col. 17, lines 32-62, with reference to its Example 1:

> Unlabeled B1 predosing was performed to assess the effect of such pre-dosing on the distribution of subsequently administered unlabeled antibody to tumors through partial or complete presaturation of non-specific binding sites and/or reservoirs of non-malignant B cells … . Predosing consistently prolonged blood and whole-body clearance of radioisotope compared to clearance without predosing … .

In the methods of the invention of the Kaminski patent, the "pre-doses" of unlabeled anti-CD20 antibody are not administered to achieve a therapeutic effect. Instead, the unlabeled antibody is administered to facilitate the therapeutic effect of the subsequently administered radiolabeled antibody – in effect, it is an adjuvant. The patent does not describe or suggest any method in which CLL can be treated using an amount of an unlabeled anti-CD20 antibody that is effective to treat the CLL, as required by new independent claims 29, 34, 55, 60, and 94.

Rejection under 35 U.S.C. § 103(a)

Claims 4, 5, 8, 11, 13, 14, 16, and 28 were rejected under § 103(a) over the combination of Kaminski '365; McLaughlin (*J. Clin. Oncol.*, 1998); Anderson, U.S. Patent No. 6,682,734; and Pouletty, EP 0 510 949 A2. For the reasons that follow, the references, whether taken singly or in combination, do not support a *prima facie* case of unpatentability as to any of claims 29-94.

The line of reasoning set forth in the Office action mailed on 7 July 2005 is that the Kaminski patent discloses the use of anti-CD20 antibodies to treat CLL, and the McLaughlin, Anderson, and Pouletty references provide disclosure that addresses various limitations of previously presented dependent claims. For the reasons discussed above, however, Kaminski does not in fact describe, nor does it suggest, any treatment of CLL using an anti-CD20 antibody. Of course, it was known at the time of the invention that certain CD20-positive B cell malignancies could be treated with anti-CD20 antibodies; CLL is a B cell tumor; and as Kaminski reports, at least some tumor cells from CLL patients are CD20-positive. Even coupled with the generic suggestion in the Kaminski reference to treat B cell tumors with radiolabeled antibodies directed against B cell antigens, this information does not render any of the present claims obvious within the meaning of § 103.

B cell tumors are not all the same. The broad class includes a variety of distinct diseases. While such diseases share some common features, they are characterized by different origins, histologies, clinical symptoms, and disease progressions. In view of the divergent characteristics of different B cell malignancies, one of ordinary skill would not reasonably have expected that a therapy shown to be effective in one type of tumor would be similarly effective in another type of tumor.

The research literature provides evidence regarding differences between types of B cell malignancies. A post-filing review article by Cogliatti *et al.*, "Who is *WHO* and what was *REAL*?," *Swiss Med. Weekly* 132: 607-17 (2002) (copy attached), for example, describes the unique disease characteristics of several tumors. Compare, *e.g.*, the characteristics of CLL in Table 2 (page 611) with the other diseases in Tables 2 and 3 (pages 611-612). A more recent review by Staudt *et al.*, "The Biology of Human Lymphoid Malignancies Revealed by Gene Expression Profiling" (available in manuscript from pubmedcentral at NIH (copy attached); published at *Adv. Immunol.* 87: 163-208 (2005)) explains that "genomic technology has revealed

that existing diagnostic categories [of lymphoid tumors] are comprised of multiple molecularly and clinically distinct diseases." The article teaches that through genomic profiling, various B cell lymphomas are shown to arise due to unrelated genetic defects. The differences in the molecular defects imply that each type of lymphoma will respond best to particular treatments.

The present application explains why it would not have been obvious to treat CLL with an anti-CD20 antibody. Paragraph 0130 states:

> This discovery [that hematologic certain malignancies including CLL may be effectively treated by the administration of a therapeutic anti-CD20 antibody] is surprising notwithstanding the reported great success of RITUXAN® (rituximab) for the treatment of relapsed and previously treated low-grade non-Hodgkin's lymphoma. In particular, this discovery is surprising given the very high numbers of tumor cells observed in such patients and also given the fact that such malignant cells, e.g., CLL cells, typically do not express the CD20 antigen at the high densities which are characteristic of some B-cell lymphomas, such as relapsed and previously-treated low-grade non-Hodgkin's lymphomas. Consequently, it could not have been reasonably predicted that the CD20 antigen would constitute an appropriate target for therapeutic antibody therapy of such malignancies.

This view is confirmed by a review article published at about the same time as the filing of the provisional application to which this application claims priority. Multani *et al.*, "Monoclonal Antibody-Based Therapies for Hematologic Malignancies," *J. Clin. Oncol.* 16(11): 3691-3710 (1998) (copy attached), states (page 3693, col. 1; emphasis added):

> The CD20 antigen is nearly an ideal target for unconjugated [monoclonal antibody] therapy because it is not expressed on precursor B or stem cells, but <u>is found in high density</u> on mature B cells (normal and malignant), with the exception of plasma cells.

CD20 is not expressed at "high density" on CLL cells. This fact is confirmed by an article by Almasri *et al.*, "Reduced Expression of CD20 Antigen as a Characteristic Marker for Chronic Lymphocytic Leukemia," *Am. J. Hematol.* 40: 259-63 (1992) (copy attached). Accordingly, one of ordinary skill would <u>not</u> consider that CD20 "is nearly an ideal target" for antibody therapy in CLL patients.

A post-filing article further supports this reasoning. Herold *et al.*, "Successful treatment and re-treatment of resistant B-cell chronic lymphocytic leukemia with the monoclonal anti-

CD20 antibody rituximab," *Ann. Hematol.* 79: 332-335 (2000) (copy attached), explains (page 333, col. 2) why the published literature (including reports of adverse reactions to rituximab in CLL patients) does not lead one of skill to expect that an anti-CD20 antibody would provide therapeutic benefit in CLL in the same manner seem in other lymphomas:

> Since trials reported to date have excluded classical B-cell chronic lymphocytic leukemia, there is no data or experience regarding the potential of rituximab for the treatment of B-CLL. The density of CD20 expression on most B-CLL cells is lower than in other B-cell lymphomas, suggesting B-CLL is less susceptible to anti-CD20 treatment.

The McLaughlin, Anderson, and Pouletty references supply neither motivation nor enabling teachings that would lead one of ordinary skill to practice the claimed invention.

The only mention of CLL in the *Journal of Clinical Oncology* paper by McLaughlin *et al.* is found in the description of the eligibility criteria for the clinical trial it describes. CLL patients were specifically excluded from the trial. See McLaughlin at page 2828, col. 1:

PATIENTS AND METHODS

Eligibility

Adult patients with relapsed low grade or follicular B-cell lymphoma, histologically confirmed and positive for CD20, were eligible. Patients with chronic lymphocytic leukemia (lymphocytes $> 5 \times 10^9$/L) were excluded.

In view of the categorical exclusion of CLL patients from the trial, McLaughlin cannot be taken to teach that any of the procedures or dose regimens it describes would be suitable for treating CLL.

The '734 patent to Anderson likewise does not describe the treatment of any CLL patients. The clinical experiments described in that patent are limited to treatments of low-grade and follicular non-Hodgkin's lymphoma. Because these types of NHL have different clinical characteristics from CLL, the teachings in Anderson do not suggest to one of ordinary skill whether or how CLL could be treated with an anti-CD20 antibody.

Lastly, the published EP application of Pouletty does not concern the treatment of CLL or the use of anti-CD20 antibodies. The disclosure relates to the generic concept of fusion proteins

involving cell-binding moieties and immunomodulatory agents. Fusions comprising immunoglobulin components are mentioned generically, but none are exemplified. The fusions are said to be useful to treat any of a comprehensively broad roster of disease categories. As described at col. 5, lines 27-28, such categories include "neoplasia, such as carcinomas, leukemias, lymphomas, sarcomas, melanomas, etc." There is no mention of even the broad subclass of B cell malignancies, let alone CLL. The Pouletty application provides no teachings that would suggest the use of a combination of any particular cell-binding moiety and an immunomodulatory agent for treating CLL to one of ordinary skill in the art.

In sum, the generic teachings of the references that relate to the treatment of cancer broadly do not suggest any method of treating CLL, nor would they convey a reasonable expectation of success to one of ordinary skill in an attempt to treat that disease. Accordingly, the examiner has failed to set forth a *prima facie* case of unpatentability.

Conclusion

For the reasons set forth above, applicant respectfully requests that the examiner withdraw the rejections set forth in the Office action mailed 7 July 2005 and allow the claims now pending.

The examiner is invited to contact the undersigned attorney should she have any questions concerning the application.

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~~TREATMENT OF HEMATOLOGIC MALIGNANCIES ASSOCIATED WITH CIRCULATING TUMOR CELLS USING CHIMERIC ANTI-CD20 ANTIBODY~~

TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA USING ANTI-CD20 ANTIBODIES

RELATED APPLICATIONS

[0010] This application claims benefit ~~of priority~~ under 35 U.S.C. § 119(e) to provisional application Serial No. 60/107,658, filed November 9, 1998, which is incorporated by reference in its entirety herein.

FIELD OF THE INVENTION

[0020] The present invention is directed to the treatment of hematologic malignancies associated with high numbers of circulating tumor cells by the administration of a therapeutically effective amount of a chimeric or humanized antibody that binds to the B-cell surface antigen Bp35 (CD20).

BACKGROUND OF THE INVENTION

[0030] The use of antibodies to CD20 as diagnostic and/or therapeutic agents for B-cell lymphoma has previously been reported. CD20 is a useful marker or target for B-cell lymphomas as this antigen is expressed at very high densities on the surface of malignant B-cells, i.e., those B-cells wherein unabated proliferation can lead to B-cell lymphomas.

[0040] CD20 or Bp35 is a B-lymphocyte-restricted differentiation antigen that is expressed during early pre-B-cell development and remains until plasma cell differentiation. It is believed that the CD20 molecule may

regulate a step in the B-cell activation process which is required for cell cycle initiation and differentiation. Moreover, as noted, CD20 is expressed at very high levels on neoplastic ("tumor") B-cells.

[0050] Previous reported therapies involving anti-CD20 antibodies have involved the administration of a therapeutic anti-CD20 antibody either alone or in conjunction with a second radiolabeled anti-CD20 antibody, or a chemotherapeutic agent.

[0060] In fact, the Food and Drug Administration has approved the therapeutic use of one such therapeutic anti-CD20 antibody, RITUXAN® (rituximab), for use in treatment of relapsed and previously treated low-grade non-Hodgkin's lymphoma (NHL). Also, the use of RITUXAN® (rituximab) in combination with a radiolabeled murine anti-CD20 antibody has been suggested for the treatment of B-cell lymphoma.

[0070] However, while anti-CD20 antibodies and, in particular, RITUXAN® (rituximab) have been reported to be effective for treatment of B-cell lymphomas, such as non-Hodgkin's lymphoma, it would be beneficial if effective antibody treatments for other malignancies could be developed. More specifically, it would be beneficial if anti-CD20 antibodies could be used for treating other types of malignancies.

BRIEF DESCRIPTION OF THE INVENTION

[0080] Toward that end, the present inventors have developed a novel treatment for hematologic malignancies characterized by a high number of tumor cells in the blood involving the administration of a therapeutically effective amount of an anti-CD20 antibody. In the preferred embodiments, such anti-CD20 antibody will comprise a chimeric, humanized, or human anti-human CD20 antibody. Examples of such hematologic malignancies include B-pro-lymphocytic leukemia

(B-PLL), chronic lymphocyte leukemia (CLL), and transformed non-Hodgkin's lymphoma.

[0090] Thus, it is an object of the invention to provide a novel treatment for hematologic malignancies comprising the administration of an anti-CD20 antibody.

[0100] It is a more specific object of the invention to provide a novel treatment for B-prolymphocytic leukemia (B-PLL), chronic lymphocytic leukemia (CLL) or transformed non-Hodgkin's lymphoma comprising the administration of an anti-CD20 antibody.

[0110] It is an even more specific object of the invention to treat B-prolymphocytic leukemia (B-PLL) or chronic lymphocytic leukemia (CLL) comprising administration of a therapeutically effective amount of RITUXAN® (rituximab).

DETAILED DESCRIPTION OF THE INVENTION

[0120] The invention involves the discovery that hematologic malignancies and, in particular, those characterized by high numbers of tumor cells in the blood may be effectively treated by the administration of a therapeutic anti-CD20 antibody. These malignancies include, in particular, CLL, B-PLL and transformed non-Hodgkin's lymphoma.

[0130] This discovery is surprising notwithstanding the reported great success of RITUXAN® (rituximab) for the treatment of relapsed and previously treated low-grade non-Hodgkin's lymphoma. In particular, this discovery is surprising given the very high numbers of tumor cells observed in such patients and also given the fact that such malignant cells, e.g., CLL cells, typically do not express the CD20 antigen at the high densities which ~~is~~ are characteristic of some B-cell lymphomas, such as relapsed and previously-treated low-grade non-Hodgkin's lymphomas.

Consequently, it could not have been reasonably predicted that the CD20 antigen would constitute an appropriate target for therapeutic antibody therapy of such malignancies.

[0140] Treatment of hematologic malignancy, such as CLL, B-PLL and transformed non-Hodgkin's lymphoma, according to the invention will comprise the administration of a therapeutically effective amount of an anti-CD20 antibody, which administration may be effected alone or in conjunction with other treatment(s), e.g., chemotherapy, radiotherapy (e.g., whole body irradiation, or treatment with radiolabeled antibodies). In addition, combination therapy with cytokines may be useful to upregulate CD20 on the surface of the lymphoma cells.

[0150] In the preferred embodiment, the anti-CD20 antibody will bind CD20 with high affinity, i.e., ranging from 10^{-5} to 10^{-9} M. Preferably, the anti-CD20 antibody will comprise a chimeric, primate, PRIMATIZED®, human, or humanized antibody. Also, the invention embraces the use of antibody fragments, e.g., Fab's, Fv's, Fab's, $F(ab)_2$, and aggregates thereof.

[0160] A chimeric antibody is intended to refer to an antibody with non-human variable regions and human constant regions, most typically rodent variable regions and human constant regions.

[0170] A PRIMATIZED® antibody refers to an antibody with primate variable regions, e.g., ~~CDR's~~ complementarity-determining regions (CDRs), and human constant regions. Preferably, such primate variable regions are derived from an Old World monkey.

[0180] A humanized antibody refers to an antibody with substantially human framework and constant regions, and non-human ~~complementarity-determining regions (~~ CDRs ~~)~~.

"Substantially" refers to the fact that humanized antibodies typically retain at least several donor framework residues (of non-human parent antibody from which CDRs are derived).

[0190] Methods for producing chimeric, primate, PRIMATIZED®, humanized, and human antibodies are well known in the art. See, e.g., U.S. Patent No. 5,530,101, issued to Queen et al, U.S. Patent No. 5,225,539, issued to Winter et al, U.S. Patent ~~s~~ Nos. 4,816,397 and 4,816,567, issued to Boss et al, and Cabilly et al, respectively, all of which are incorporated by reference in their entirety.

[0200] The selection of human constant regions may be significant to the therapeutic efficacy of the subject anti-CD20 antibody. In the preferred embodiment, the subject anti-CD20 antibody will comprise human, gamma 1, or gamma 3 constant regions and, more preferably, human gamma 1 constant regions. The use of gamma 1 anti-CD20 antibodies as therapeutics is disclosed in U.S. Patent No. 5,500,362, issued to Robinson et al.

[0210] Methods for making human antibodies are also known and include, by way of example, production in SCID mice, and *in vitro* immunization.

[0220] As noted, a particularly preferred chimeric anti-CD20 antibody is RITUXAN® (rituximab), which is a chimeric gamma 1 anti-human CD20 antibody. The complete ~~amino acid and corresponding~~ nucleic acid sequence ~~for~~ encoding this antibody and the corresponding amino acid sequences of the heavy chain and light chain variable domains may be found in U.S. Patent No. 5,736,137, which is incorporated by reference in its entirety. This antibody, which is produced in a proprietary CHO cell expression system commercialized by IDEC Pharmaceuticals Corporation, ~~is~~ may be made by a CHO cell transfectoma comprising the vector DNA present in the *E. coli* host cell ~~which was~~ deposited on November 4, 1992,

under the provisions of the Budapest Treaty at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209, under accession no. ~~(ATCC~~ 69119 ~~)~~. This ~~cell line~~ deposit was determined to be viable and will be replaced should it become non-viable during the term of deposit. This ~~cell line~~ deposit was made irrevocably available upon issuance of ~~the~~ U.S. Patent No. 5,736,137 ~~patent~~ and is available without restriction from the ATCC. This ~~cell line~~ deposit will also be available without restriction during the lifetime of any patent that may issue based on this application.

[0230] The subject anti-CD20 antibody will be administered by various routes of administration, typically parenteral. This is intended to include intravenous, intramuscular, subcutaneous, rectal, and vaginal ~~, and~~ administration, with intravenous infusion being preferred.

[0240] The anti-CD20 antibody will be formulated for therapeutic usage by standard methods, e.g., by addition of pharmaceutically acceptable buffers, e.g., sterile saline, sterile buffered water, propylene glycol, and combinations thereof.

[0250] Effective dosages will depend on the specific antibody, condition of the patient, age, weight, or any other treatments, among other factors. Typically effective dosages will range from about 0.001 to about 30 mg/kg body weight, more preferably from about 0.01 to 25 mg/kg body weight, and most preferably from about 0.1 to about 20 mg/kg body weight.

[0260] Such administration may be effected by various protocols, e.g., weekly, bi-weekly, or monthly, dependent on the dosage administered and patient response. Also, it may be desirable to combine such administration with other treatments, e.g., radioactive therapy, both targeted and

non-targeted, chemotherapies, and lymphokine or cytokine administration, e.g., interleukins, interferons, ~~TNF's~~ TNFs, colony stimulating factors, etc.

[0270] Typically, treatment will be effected weekly, for about 2 to 10 weeks, more typically about 4 weeks. A particularly preferred dosage regimen will comprise administration of about 375 ~~mg/kg~~ mg/m^2 weekly for a total of four infusions. Also, stepped-up dosing schedules may be even more preferable.

[0280] If radiation is used in conjunction with the therapeutic anti-CD20 antibody, it is preferred that an yttrium-labeled anti-CD20 antibody be utilized, such as the one disclosed in U.S. Patent No. 5,736,137, incorporated by reference in its entirety herein. This antibody, $[^{90}Y]$-2B8-MX-DTPA, has reported efficacy in the treatment of B-cell lymphoma. The ~~cell line~~ hybridoma that produces the 2B8 antibody ~~has also been~~ was deposited at the American Type Culture Collection under accession no. HB 11388 on June 22, 1993, under the provisions of the Budapest Treaty, and was made irrevocably available upon issuance of US Patent No. 5,736,137, without any restrictions. This ~~cell line~~ hybridoma was found to be viable and ~~shall similarly~~ will be replaced during the lifetime of any patent that issues based on this application, should it become non-viable.

[0290] A particularly preferred chemotherapeutic regimen that may be used in conjunction with the subject antibody immunotherapy comprises CHOP ~~immunotherapy~~ chemotherapy, which comprises the administration of a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone. Other known chemotherapeutics include methotrexate, cisplatin, toremifene, and tamoxifen.

[0300] The following ~~E~~ examples are not intended, nor are they to be construed, as limiting the invention. The ~~E~~ examples

are intended to provide clinical evidence in support of the efficacy of the invention.

EXAMPLE 1

[0310] Two patients in whom we noted rapid reduction of blood tumor cells, which was associated with severe pulmonary infusion-related toxicity and thrombocytopenia, were studied. Also, two additional patients were collected from physician-submitted reports of adverse events related to RITUXAN® (rituximab) treatment. Pretreatment characterization of these patients included a median age of 60 years (range 26-73) with the diagnosis of B-prolymphocytic leukemia (B-PLL), chronic lymphocytic leukemia (CLL), or transformed non-Hodgkin's lymphoma. All of these patients had elevated leukocyte counts as a consequence of blood tumor involvement, bulky adenopathy and organomegaly. All four patients developed a unique syndrome of severe infusion-related reactions characterized by fever, rigors, bronchospasm with associated hypoxemia, requiring temporary cessation of RITUXAN® (rituximab) therapy. Concurrent with these symptoms, a rapid decrement in circulating tumor cell load (mean pretreatment 98 x ~~10^9 per L~~ 10^3L; range 73-132 vs. mean post-treatment 11 x ~~10^9 per L~~ 10^3L; range ~~3.7~~ 37-24.6) with mild electrolyte evidence of rapid tumor lysis was observed. Thrombocytopenia, a finding not commonly associated with RITUXAN® (rituximab) therapy, was noted in all four patients (mean pretreatment 145 x ~~10^9 per L~~ 10^3L; range 57-277 vs. mean post-treatment ~~56~~ Sn x 10^9/L; range 2-120), requiring transfusion in one case. Symptoms of this syndrome required hospitalization but resolved with supportive care. Subsequent RITUXAN® (rituximab) treatment were well tolerated in all patients.

[0320] Two subsequent patients with CLL have been treated with high blood tumor counts utilizing stepped-up dosing (100 mg

~~/m²~~ day 1 followed by the rest of therapy on day ~~2~~ 1) with demonstrated efficacy, thrombocytopenia but minimal infusion-related toxicity. RITUXAN® (rituximab) administration in patients with hematologic malignancies who have blood tumor cell involvement may be associated with a higher frequency of severe initial infusion-related reactions and thrombocytopenia mandating careful clinical monitoring. Given the preliminary activity of RITUXAN® (rituximab) in these patients, future studies in CLL and PLL, utilizing a stepped-up dosing schedule, ~~is~~ are to be conducted.

EXAMPLE 2

[0330] Unlabeled immunoglobulins (monoclonal antibodies, Mabs) are attractive for the treatment of NHL as they may: mediate cytotoxicity with complement (CDC) or effector cells (ADCC); effect apoptosis; be less toxic, less immunogenic and possibly more effective than toxin- or drug-conjugated Mabs; not require the complex procedures needed for radiolabeled Mab therapy (radioimmunotherapy, RIT), and not produce the myelosuppression typical of high-dose RIT.

[0340] Until recently, use of Mabs in the treatment of hematologic malignancies has been limited. However, the chimeric anti-CD20 Mab, RITUXAN® (rituximab), has a low toxicity profile and significant clinical efficacy and is now approved by the U.S. Food and Drug Administration (November 1997 ~~US FDA 11/97; EU 6/98~~) and in the E.U. (June 1998) for the treatment of relapsed or refractory, low-grade or follicular (~~R=~~ R-LG/F) NHL. In a single-agent phase III clinical trial ~~(PIII)~~, of 166 patients with R-LG/F NHL treated with RITUXAN® (rituximab) at 375 mg/m² weekly for four infusions (study 102-05), the overall response rate (ORR) was 48% (6% complete response (CR) and 42% partial response (PR)). Median time to progression for responders

was 13.1 months and duration of response was 11.2 months. Median circulating B-lymphocyte counts dropped to zero following the first dose. CD3, CD4, CD8 and NK cell counts remained unchanged. B-cell recovery in peripheral blood began at 6-9 months and was complete by 9-12 months. No significant changes in serum complement levels were noted. The mechanism for action remains unresolved with CDC, ADCC, apoptosis and/or others being considered. In spite of the absence of a clinical/ laboratory correlation, the contribution of CDC cannot be discounted. We have seen a correlation between higher absolute NK cell count at baseline and response to the Mab.

Cell Type	# Patients CR+PR	Abs. Count	# Patients NR	Abs. Count	P-value
NK	98	180	15	98	0.02
MK+ANC	98	185	15	102	0.02
ANC	101	3.7	15	3.4	0.40
CD3+	98	761	15	576	0.37
Platelets	101	187	15	206	0.32
Note: N = 166 patients from study 102-05, and 37 from 102-06. Abs. Count: NK, CD3 = cells/~~mm3~~ mm^3; ANC, Pts. = cells x $10^3/mm^3$. P value for the difference between Abs. Counts.					

[0350] ADCC may be an important mechanism for the clinical activity seen in patients treated with RITUXAN® (rituximab). Agents which enhance effector cell number and activity may synergize with the Mab. Studies of RITUXAN® (rituximab) in combination with cytokines, e.g., Il-2, G-CSF, GM-CSF, INF, are also ongoing.

EXAMPLE 3

Phase I/II Study of RITUXAN® (rituximab) in CLL

[0360] RITUXAN® (rituximab) is a monoclonal antibody targeting CD20 that has significant activity in the treatment of low-grade lymphoma (LGL). When given at a dosage of 375 mg/m^2 weekly for four weeks the response rate in relapsed patients ~~(PTS)~~ was 43% (~~McLaughlin~~ McClaughlin et al., KOO, Vol. 14, ~~(~~ 1998) ~~J Clin Oncol 16(8):2825 33)~~. Patients with small lymphocytic lymphoma (SLL) had lower response rates (13%) than patients with other subtypes of LGL and lower serum levels of RITUXAN® (rituximab). Reduced response seen in SLL could be related to lower density of CD20 antigen and/or higher circulating B-cell counts. Both factors would be expected to impact (negatively) on response seen in CLL.

[0370] In an attempt to maximize activities in CLL we are conducting a Phase I/II study. All patients receive a first dose of 375 mg/m^2 to minimize infusion ~~relapsed~~ related side effects. Subsequent weekly dosages (3) remain the same but are given at an increased dose level. Sixteen patients have been treated at dosages of 500-1500 mg/m^2. Median age was 66 years (range, 25-78). Eighty-one percent had end-stage III-IV disease. Median white blood cell count was 40 x 10^9/L (range, 4-200), Hgb 11.6 g/dl (range, 7.7-14.7), platelets 75 x 10^9/L (range, 16-160), median β_2 ~~immunoglobulin~~ microglobulin was 4.5 mg/L (range, 3.1-9.2). Median numbers of prior therapies was 2.5 (range 1-9). Sixty percent of patients were refractory to treatment. Two patients developed severe hypertension with the first dose (375 mg/m^2); another one received further therapy. Toxicity at subsequent escalated dosages has been mild although no patient at the 1500 mg/m^2 dose level has been fully evaluated. Eight patients have completed therapy (4 at 500 mg/m^2, 3 at 650 mg/m^2, 1 at 825 mg/m^2). One patient treated

at 560 mg/m^2 achieved full remission. One patient has progressive lymphocytosis on treatment and all other patients had reduction in peripheral blood lymphocytosis but less effect on lymph nodes. Dose escalation studies are ongoing.

EXAMPLE 4

Use of ~~c~~Cytokines to ~~u~~Upregulate the ~~e~~Expression of CD20

[0380] Another approach to improving response in CLL patients is to upregulate the CD20 antigen using cytokines. In an *in vitro* study, mononuclear cells from CLL patients were incubated for 24 hours with various cytokines. Flow cytometry results showed significant up-regulation by IL-4, GM-CSF, and TNF-alpha. (Venugopal P, Sivararnan S, Huang X, Chopra H, O'Brein T, Jajeh A, Preisler H. Upregulation of CD20 expression in chronic lymphocytic leukemia (CLL) cells by *in vitro* exposure to cytokines. *Blood* 1998; 10:247a.) In fact, recent data suggest~~s~~ that the upregulation of CD20 observed on CLL cells may be limited to tumor cells (Venogopal et al. Poster - PanPacific Lymphoma meeting, June 1999. Cytokine-induced upregulation of CD20 antigen expression in chronic lymphocytic leukemia (CLL) cells may be limited to tumor cells). Preliminary data also suggest that interferon alpha also upregulates CD20 on CLL cells after only 24 hours when applied at a concentration of 500 to 1000 U/ml.

[0390] Thus, by administering certain cytokines to CLL patients prior to or concurrently with administration of RITUXAN® (rituximab), the expression of CD20 on the surface of malignant B-cells may be upregulated, thereby rendering CD20, as well as other cell surface markers such as CD19, a more attractive target for immunotherapy.

[0400] A collaborative study has been initiated to test for optimal cytokine doses for CD20 upregulation in vivo. The study protocol involves treating ten patients initially with GM-CSF at 250 mcg/m^2 SQ QD X 3, ten patients with IL-4 mcg/kg SQ QD X 3, and ten patients with G-CSF at 5 mcg/kg SQ QD X 3. Mononuclear cells will be separated by ~~Ficon~~ FICOLL® (sucrose-epichlorohydrin copolymer) Hypaque centrifugation for apoptotic studies to determine if upregulation of CD20 translates to enhanced killing of tumor cells by RITUXAN® (rituximab).

EXAMPLE 5

Combination Antibody and Chemotherapy Protocol

[0410] Antibody treatment of CLL can be combined with other conventional chemotherapeutic treatments known to be useful for the treatment of CLL. The most frequently used single agent for CLL is chlorambucil (~~leukeran~~ LEUKERAN®), given either as 0.1 mg/kg daily or 0.4 to 1.0 mg/kg every 4 weeks. Chlorambucil is often combined with oral prednisone (30 to 100 mg/m^2/d), which is useful in the management of autoimmune cytopenias. Cyclophosphamide is an alternative to chlorambucil, the usual dose being 1-2 g/m^2 every 3-4 weeks together with vincristine and steroids (e.g., COP regimen).

[0420] Various drug combinations have been used for CLL, including COP (cyclophosphamide, Oncovin, and prednisone), and CHOP (these three drugs plus doxorubicin). Fludarabine has shown an effect in the treatment of CLL, and gave an ORR of 50% in a group of patients treated with 25-30 mg/m^2/d every 3-4 weeks. See ~~O'Brien et al. (1996) Ann. Oncol. 7 Suppl. 6: S27:33; and Keating et al. (1994) Drugs 47 Suppl. 6: 39 49, (1993) Semin. Oncol. 20 (5 Suppl. 7): 13 20, and (1988) Nouv. Rev. Fr. Hematol. 30 (5 6): 461 6~~ www.

cancernetwork. com. ~~Although s~~ Some patients have been shown to be refractory for fludarabine. Such patients may also be resistant to 2-CdA because often, patients who are refractory to fludarabine are also refractory to 2-CDA (O'Brien et al. N. Engl. J. Med. 330: 319-322 (1994)).

[0430] Hence, anti-CD20 antibody therapy will be particularly useful for patients who are refractory or who have relapsed after treatment with chemotherapeutic drugs. RITUXAN® (rituximab) therapy may also be combined with radiotherapy in these patients. TBI with a low fraction size of 15 cGy to total doses of 75 to 150 cGy has been shown to be effective in about one-third of patients.

[0440] A Phase II trial is currently being conducted by CALGB in CLL patients. RITUXAN® (rituximab) and fludarabine are administered concurrently, followed by RITUXAN® (rituximab) consolidation versus fludarabine induction followed by RITUXAN® (rituximab). The goals of the study are (1) to determine in fludarabine treated CLL patients the complete response (CR) rate and toxicity profile of concurrent and consolidative RITUXAN® (rituximab) therapy (Arm I) and of consolidative RITUXAN® (rituximab) therapy (Arm II); (2) to assess the CR rate in patients receiving concurrent therapy with RITUXAN® (rituximab) and fludarabine (the inductive phase of Arm I); (3) to assess the frequency of conversion of a partial response (PR) to a CR or stable disease to either PR or CR in CLL patients receiving consolidative therapy with RITUXAN® (rituximab); (4) to follow the effects of therapy with RITUXAN® (rituximab) and fludarabine on the immunologic markers CD4, CD8, IgG, IgA and IgM; and (5) to examine progression-free survival and overall survival in Arms I and II.

[0450] Although the present invention has been described in some detail by way of illustration and example, for purposes of clarity and understanding it will be apparent that

certain changes and modifications may be practical within the scope of the appended claims.

ABSTRACT OF THE DISCLOSURE

~~A method of treating hematologic malignancies associated with a high number of circulating tumor cells by the administration of a therapeutic chimeric anti-CD20 antibody. These malignancies include in particular B-prolymphocytic leukemia (B-PLL), chronic lymphocytic leukemia (CLL), and transformed non-Hodgkin's lymphoma.~~

Chronic Lymphocytic Leukemia (CLL) may be treated with antibodies directed against the CD20 antigen.